Distribution of calcium oxalate crystals in the cypselar walls in some members of the Compositae and their taxonomic significance

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Abstract

Shape, structure and distribution of calcium oxalate crystals and druses are important from the taxonomic point of view because they are not universally present in all parts of the plant organ, but instead they are confined to specific parts and certain plant tissues in some restricted taxa only. Actually the distribution and structure of such crystals appear to be genetically controlled and are quite stable and have paramount taxonomic value.

The authors have scrutinized the mature cypselar walls of 93 genera and 141 species belonging to 19 tribes of 4 subfamilies of the family Compositae and noted the presence of oxalate crystals in 22 genera (*Anthemis*, *Arctium, Aster, Baccharoides, Bothriocline, Brachycome, Buphthalmum, Carpesium, Catananche, Cirsium*, *Craspedia*, *Elephantopus*, *Emilia*, *Gaillardia, Inula*, *Linzia*, *Polydora, Steirodiscus, Tanacetum, Tripleurospermum*, *Vernonanthura,* and *Vernonia*) and 27 species only. These crystals are usually of the common rectangular type but seldom druses (from cross sectional view of the cypselar wall). The distribution of crystals is also very specific. In cases they are distributed in the epicarpic zone of cypselas (*Aster thomsonii, Brachycome heterodonta, Carpesium cernuum, Carpesium nepalense, Inula ensifolia, Buphthalmum speciosissimum*) they are also visible in dry condition from the scanning electron photographs of cypselas. In other instances they are only observable from histological structure. In *Anthemis tinctoria, Arctium lappa, Bothriocline laxa, Brachycome campylocarpum, Catananche caerulea, Elephantopus scaber* and *Tanacetum macrophyllum* crystals are found in different parts of the mesocarpic zone of pericarp, whereas in different species of *Vernonia* s.lat. they are noted from other zones of pericarp. They are restricted to testal region only in *Tripleurospermum maritimum* and

Steirodiscus tagetes. Seldom, they are also found both in the pericarpic zone and the testal zone as in *Buphthalmum salicifolium*.

From this study it is also notable that the formation of calcium oxalate crystals may be antagonistic to the formation of phytomelanin pigment, because all investigated taxa with a phytomelanin layer (belonging to the tribes Eupatorieae, Heliantheae, Coreopsideae and Tageteae) do not possess crystals.

Keywords: Calcium oxalate crystals, cypselar wall, Compositae.

Introduction

The presence of calcium oxalate (CaOx; CaC_2O_4) crystals in flowering plants is a phenomenon that appears to be more or less widespread in different plant parts, such as leaves, stems, roots, floral parts, fruits and seeds. These crystals may be of two types, extracellular and intracellular. The intracellular granular deposits of calcium oxalate have been reported initially in about 160 angiospermic families, but most prevalently in the Amaranthaceae, Rubiaceae and Solanaceae (Metcalfe & Chalk 1950, 1983). Later, these crystals have been recorded from over 215 families including Compositae (Franceschi & Nakata 2005). The Compositae are regarded as "Potassium plants" with a small amount of soluble calcium and high amount of K:Ca ratios (Kinzel 1982, White & Broadley 2003).

Occurrence of calcium oxalate crystals from the pericarp of cypsela in Compositae had been recorded already by Hanausek (1911) from the genus *Carthamus*. However, the pioneer and most significant paper in this field was the work of Dormer (1961), who described the shape and distribution of calcium oxalate crystals in the ovaries of certain members of the tribe Cardueae, viz., *Onopordum, Centaurea, Carthamus* and *Cirsium*. The crystals were found in certain restricted taxa and in specific crystalliferous tissues, which usually occur in the inner part of ovary wall. According to Dormer the distribution and shape of such crystals appear to be genetically controlled and hence taxonomically significant.

In a second paper Dormer (1962) also discussed the taxonomic significance of crystal forms in 112 species of *Centaurea*, where six different types of crystals were recorded and a correlation between the crystalline structures and palynological characters could be established. Drury & Watson (1965) noted the taxonomic value of the crystals in the ovary and achene wall of the genus *Senecio* (s.lat.) and categorized four types of calcium oxalate crystals on the basis of their length and breadth ratio. Gochu (1973) demonstrated that some further species of *Centaurea* have calcium oxalate crystals. Nordenstam & El-Ghazaly (1977) found two types of crystals in the Centaureinae, viz. the *Centaurea* (short and broad crystals with rounded corners) and the *Amberboa* type (elongate hexagonal crystals with sharp corners). ROBINSON & KING (1977) stated that calcium oxalate crystals are absent in the achene walls within the tribe Eupatorieae. Importance of crystals in the achenial wall within tribe Senecioneae was noted by the second author (NORDENSTAM 1977, 1978).

PANDEY et al. (1978) observed that a rod-shaped crystal exists in all crystalliferous cells of integument in some members of the tribe Cichorieae, but later the crystals become depleted in the seed coat. Therefore, the form and distribution of crystals are not always taxonomically valuable features as far as cypselar anatomy is concerned. The same type of single crystals was reported by CIHAN & PALSER (1982) from *Cichorium intybus*. Clustered druses and rectangular crystals were found in the subepidermal cells of seed coat in *Carthamus oxyacantha* and *Centaurea cyanus* in the tribe Cardueae (SINGH & PANDEY 1984). Presence or absence of druses in the epidermal cells of pericarp proved useful for delimitation of taxa of some members of the tribe Anthemideae (KÄLLERSJÖ 1985, 1990). Importance of different forms of crystals has been mentioned by AHMED et al. (1986) in the genus *Aster* (tribe Astereae).

The value of crystals for identification and delimitation of taxa was noted by REESE (1989) in his carpological work on tribes Calenduleae and Arctotideae. He observed that the crystals are either found in the subepidermal cells of pericarp (e.g., *Arctotheca calendula*), or in subepidermal cells of testa (e.g., *Cuspidia cernua*), or in the subepidermal cells of both testa and pericarp (e.g., *Heterolepis aliena*, a taxon of uncertain tribal position).

The distribution, type and morphology of calcium oxalate crystals in different floral organs of *Helianthus annuus* and *H. tuberosus* have been studied by MERIC & DANE (2004). Mode of distribution and shape of crystals have been studied in two taxa of *Serratula* and *Synurus* in the tribe Cardueae by ZAREMBO & BOYKO (2008). Recently MERIC (2008, 2009) has investigated the morphology and distribution of calcium oxalate crystals in four taxa of tribe Astereae, viz., *Conyza canadensis, C. bonariensis, Aster squamatus* and *Bellis perennis*, with the help of both light microscopic and SEM studies.

MERXMÜLLER &GRAU (1977) in a carpological study of the Inuleae found that 12 genera of the *Inula* group were characterized by the presence of a large oxalate crystal in each epidermis cell. As pointed out by ANDERBERG this single epicarpic crystal is a strong synapomorphy of the Inuleae-Inulinae (ANDERBERG et al. 2005, ANDERBERG 2009).

The principal aims of the present study are (i) to determine the presence or absence of calcium oxalate crystals in mature cypselar wall, (ii) to elucidate the exact location of these crystals in pericarp or in testa or in both, (iii) to assess the types of crystals, (iv) to evaluate the taxonomic significance of crystal structure in plant tissues, (v) to update the status of our knowledge on the presence of calcium oxalate crystals in cypselar wall in Compositae.

Up till now, there is limited available information on the existence of calcium oxalate crystals in mature cypselar wall for the majority of taxa belonging to the Compositae. The distribution and structure of oxalate crystals have been reported by different researchers during revisionary study or histological work on a few and sometimes randomly selected taxa.

Materials and Methods

We studied cross-sectioned dried mature cypselas from the following herbaria as indicated in Holmgren et al. (1990): AD, BRI, LISC, NSW, RB, SRGH, and Z. Some species were collected by the first author from India and deposited in the Herbarium of the Department of Botany, University of Kalyani, Kalyani – 741235, West Bengal, India, with the proposed new acronym KAL. Tribal classification of Compositae follows Funk et al. (2009). The genera and species are arranged in alphabetical sequence.

For histological observation, mature dry cypselas were rehydrated in boiling water and stored in FAA solution (Johansen 1940). For each species, five randomly selected cypselas were studied. Thin hand sections from the middle part of cypselas were stained with fast green/safranin combination after some modification (Johansen 1940) and were observed under compound research microscope.

A few cypselas were selected for SEM observation, after following standard techniques in SEM and were photographed in Philips SEM at R.S.I.C. of Bose Institute of Kolkata, West Bengal, India and Hitachi SEM at the University of Burdwan, Scientific Instrumentation Centre, Burdwan, West Bengal, India.

Sources of Cypselas with Crystals

Tribe Vernonieae – *Baccharoides calvoana* (Hook. f.) Isawumi, El-Ghazaly & B.Nord. ssp. *meridionalis* (Wild) Isawumi, El-Ghazaly & B. Nord., SRGH; G. Pope 1930. *Bothriocline laxa* N. E. Br. ssp. *laxa*, SRGH; M. Mavi 11. *Elephantopus scaber* L., RB; SN 257. *Linzia melleri* (Oliv. & Hiern) H. Rob., LISC; R. Santos 2051. *Polydora poskeana* (Vatke & Hildeb.) H. Rob., LISC; A. R. Torre & Paiva 11332. *Vernonanthura condensata* (Baker) H. Rob., RB; SN 249. *Vernonia cistifolia* O. Hoffm., SRGH; G. Pope 1931. *Vernonia petersii* Oliv. & Hiern, LISC; A. R. Torre 118. *Vernonia senegalensis* Less., LISC; Schlieben 2457.

Tribe Astereae – *Aster thomsonii* C. B. CLARKE, KAL; S. MUKHERJEE 3. *Brachycome campylocarpa* J. M. BLACK, NSW; L. HAEGI 2065. *Brachycome heterodonta* DC. var. *heterodonta*, NSW; L. HAEGI 2064.

Tribe Gnaphalieae – *Craspedia uniflora* FORST. f., AD; A. A. MUNIR 5498.

Tribe Inuleae – *Buphthalmum salicifolium* L., Z; Nr. 356. *Buphthalmum speciosissimum* ARD., Z; Nr. 357. *Carpesium cernuum* L., Z; Nr. 362. *Carpesium nepalense* LESS., KAL; S. MUKHERJEE 4. *Inula ensifolia* L., Z; Nr. 405.

Tribe Helenieae – *Gaillardia aristata* PURSH, Z; Nr. 395.

Tribe Senecioneae – *Emilia flammea* CASS., Z; Nr. 389. *Steirodiscus tagetes* (L.) SCHLTR. (*Gamolepis annua* LESS.), Z; Nr. 396.

Tribe Anthemideae – *Anthemis tinctoria* L., Z; Nr. 342. *Tanacetum macrophyllum* (WALDST. & KIT.) SCH.-BIP., Z; Nr. 373. *Tripleurospermum maritimum* (L.) W. D. J. KOCH, Z; Nr.374.

Tribe Cardueae – *Arctium lappa* L., Z; Nr. 343. *Cirsium vulgare* (SAVI) TEN., BRI; s. n., s. coll.

Tribe Cichorieae – *Catananche caerulea* L., Z: Nr. 363.

Results

The mode of distribution and types of calcium oxalate crystals in 27 species of Compositae, out of totally 141 species investigated, are summarized in Tables 1 & 2. The distributional data of calcium oxalate crystals are solely based on the studies of cross sectional configuration of mature cypselar wall as well as surface structure of the cypselar wall with the help of SEM studies. Based on our visual observations, it is not possible to determine precisely the size of crystals in each species studied. However, types and location of crystals are documented here.

Table 1. Distribution of calcium oxalate crystals in mature cypselar wall in Compositae from the present study.

Sl. No.	Genus, Species and Tribe	Types of crystals	Distribution of crystals in pericarp and testa
1.	*Anthemis tinctoria* Anthemideae (Figs. 1, 2)	Elongated, hexagonal prismatic crystals	Cypselar surface shows vertically arranged crystals in 5–12 rows in each furrow, visible after clearing of cypselar wall, mesocarpic palisade cells have crystals.
2.	*Arctium lappa* Cardueae (Fig. 3)	Prismatic crystals	Innermost mesocarpic zone of thick-walled parenchyma cells, adjacent to testa epidermis.
3.	*Aster thomsonii* Astereae (Figs. 4, 28)	Prismatic crystals	Epicarpic cells, secondary sculpture formed by visible calcium oxalate crystals, situated inside the epicarpic zone.
4.	*Baccharoides calvoana* ssp. *meridionalis* Vernonieae (Fig. 5)	Prismatic crystals	Found in basal arm of inverted "T" shaped sclerenchymatous bundle and few adjacent parenchyma cells of mesocarp.
5.	*Bothriocline laxa* ssp. laxa Vernonieae (Fig. 6)	Pentagonal to hexagonal, prismatic crystals	Outermost, sclerenchymatous mesocarpic zone (uni-layered), crystals found towards outer walls.
6.	*Brachycome campylocarpa* Astereae (Figs. 7, 29)	Druse crystals	Multi-layered mesocarpic pitted parenchyma cells of anterio-posterior lobes only.

7.	*B. heterodonta* var. *heterodonta* Astereae (Fig. 30)	Druse crystals	Secondary sculpture formed by druse-like crystals, situated inside epicarpic zone.
8.	*Buphthalmum salicifolium* Inuleae (Figs. 8, 31)	Prismatic crystals	Both epicarpic cells and testal layers.
9.	*Buphthalmum speciosissimum* Inuleae (Figs. 27, 32)	Prismatic crystals	Epicarpic cells, secondary sculpture formed by visible calcium oxalate crystals, situated inside the epicarpic zone.
10.	*Carpesium cernuum* Inuleae (Figs. 9, 33)	Prismatic crystals	Epicarpic cells.
11.	*C. nepalense* Inuleae (Figs. 10, 34)	Prismatic crystals	Epicarpic cells.
12.	*Catananche caerulea* Cichorieae (Fig. 11)	Prismatic crystals	Multi-layered, inner mesocarpic parenchymatous cells.
13.	*Cirsium vulgare* Cardueae (Fig. 12)	Prismatic crystals	Middle layer of the thin-walled parenchymatous testa cells.
14.	*Craspedia uniflora* Gnaphalieae (Fig. 13)	Prismatic crystals	Outer crusted testal epidermal layer.
15.	*Elephantopus scaber* Vernonieae (Fig. 14)	Prismatic crystals	Mesocarpic parenchymatous cells of the furrow region only.
16.	*Emilia flammea* Senecioneae (Fig. 15)	Prismatic crystals	Thick-walled testal cells.

17.	*Gaillardia aristata* Helenieae (Fig. 16)	Prismatic crystals	Outer, one-layered, thin-walled, palisade parenchyma cells of mesocarp only, crystals situated near outer wall of the cells.
18.	*Inula ensifolia* Inuleae (Fig. 17, 35)	Prismatic crystals	Epicarpic cells, secondary sculpture formed by visible calcium oxalate crystals, situated inside the epicarpic zone.
19.	*Linzia melleri* Vernonieae (Fig. 18)	Prismatic crystals	Found in mesocarpic thick-walled parenchyma cells, surrounding sclerenchyma brace of rib region.
20.	*Polydora poskeana* Vernonieae (Fig. 19)	Prismatic crystals	Thick-walled mesocarpic cells.
21.	*Steirodiscus tagetes* Senecioneae (Fig. 20)	Prismatic crystals	Testal zone.
22.	*Tanacetum macrophyllum* Anthemideae (Fig. 21)	Druse crystals	Epicarpic and mesocarpic parenchyma cells in many layers.
23.	*Tripleurospermum maritimum* Anthemideae (Fig. 22)	Prismatic crystals	Found in sclerenchyma cells of testal layer (uni-layered).
24.	*Vernonanthura condensata* Vernonieae (Fig. 23)	Prismatic crystals	In furrow region, outermost layer of parenchymatous mesocarpic cells.
25.	*Vernonia cistifolia* Vernonieae (Fig. 24)	Prismatic crystals	In furrow region, parenchymatous, thick-walled, mesocarpic cells.

26.	*Vernonia petersii* Vernonieae (Fig. 25)	Prismatic crystals	Mesocarpic, thick-walled sclerenchymatous tissue .
27.	*Vernonia senegalensis* Vernonieae (Fig. 26)	Prismatic crystals	Mesocarpic, thick-walled sclerenchymatous tissue.

End of Table 1.

Table 2. No. of studied taxa of Compositae, scrutinized for the presence or absence of CaOx crystals.

Sl. No.	Tribe	Number of genera and species with CaOx crystals	Number of genera and species without CaOx crystals
1.	Eupatorieae	-	5 genera, 7 species
2.	Vernonieae	7 genera, 9 species	5 genera, 9 species
3.	Astereae	2 genera, 3 species	4 genera, 10 species
4.	Gnaphalieae	1 genus, 1 species	-
5.	Inuleae	3 genera, 5 species	12 genera, 20 species
6.	Madieae	-	1 genus, 1 species
7.	Coreopsideae	-	5 genera, 7 species
8.	Heliantheae	-	4 genera, 5 species
9.	Helenieae	1 genus, 1 species	-
10.	Tageteae	-	1 genus, 1 species
11.	Senecioneae	2 genera, 2 species	6 genera, 16 species
12.	Anthemideae	3 genera, 3 species	4 genera, 5 species
13.	Arctotideae	-	3 genera, 3 species
14.	Calenduleae	-	2 genera, 2 species
15.	Cardueae	2 genera, 2 species	6 genera, 10 species
16.	Pertyeae	-	1 genus, 2 species
17.	Dicomeae	-	1 genus, 1 species
18.	Mutisieae	-	1 genus, 1 species
19.	Cichorieae	1 genus, 1 species	10 genera, 14 species
		22 genera, 27 species	71 genera, 114 species
	Total studied taxa	**93 genera**	**141 species**

Discussion and Conclusion

The present study shows that 22 genera out of 93 genera investigated, and 27 species out of 141 species have calcium oxalate crystals; i.e. about 24 % of the genera and 19 % of the species possess calcium oxalate crystals. There are no correlations among the tribes of Compositae on the basis of distributional and data of calcium oxalate crystals, but this study indicates that the formation of calcium oxalate crystals may be antagonistic to the formation of phytomelanin pigment, since all taxa with a phytomelanin layer (belonging to the tribes Eupatorieae, Heliantheae, Coreopsideae and Tageteae) do not possess crystals. Similar type of statement had been advocated by Robinson & King (1977) on the achene wall of the tribe Eupatorieae. Although *Gaillardia* used to belong to the Heliantheae, it is now included in the tribe Helenieae, subtribe Gaillardiinae, which has no phytomelanin layer, and this genus possesses crystalliferous tissues in pericarp.

In the mature cypselar wall there are two basic types of crystals. Predominant is the prismatic crystal type. Seldom druses are also available from the pericarpic zone (*Brachycome campylocarpa, Brachycome heterodonta* var. *heterodonta, Tanacetum macrophyllum*). Calcium oxalate crystals have been noted from cross sectional configuration of cypselar wall, i.e., pericarp associated with seed coat of cypselas. Sometimes structure of crystals is also observable in dry condition from the SEM photographs of cypselas, where crystals are distributed in the epicarpic zone of cypselas as in *Aster thomsonii, Brachycome heterodonta* var. *heterodonta, Buphthalmum salicifolium, Buphthalmum speciosissimum, Carpesium cernuum, Carpesium nepalense, Inula ensifolia*. In these taxa, secondary sculpture is formed by the visible calcium oxalate crystals, which are situated inside the cell as cell inclusion.

On the basis of present observation, the distribution of calcium oxalate crystals and druses in the pericarp and/or testa is particularly important for the tribes of Vernonieae, Astereae, Inuleae, Senecioneae, Anthemideae and Cardueae and is less important in Arctotideae, Calenduleae, Pertyeae, Dicomeae, Mutiseae and Cichorieae. On the basis of distribution of calcium oxalate crystals in different tissue region of cypselas, the studied taxa could be grouped into the following five categories:

Category I	Crystals are distributed in different tissue regions of mesocarpic zone of pericarp; viz., *Anthemis tinctoria, Arctium lappa, Baccharoides calvoana* ssp. *meridionalis, Bothriocline laxa* ssp. *laxa, Brachycome campylocarpa, Catananche caerulea, Elephantopus scaber, Gaillardia aristata, Linzia melleri, Polydora poskeana, Vernonanthura condensata* and 3 *Vernonia* spp.

Category II	Crystals are distributed only in epicarpic zone of pericarp; viz., *Aster thomsonii, Brachycome heterodonta* var. *heterodonta, Carpesium cernuum, Carpesium nepalense, Inula ensifolia* and *Buphthalmum speciosissimum.*
Category III	Crystals are distributed in testal zone; viz., *Cirsium vulgare, Craspedia uniflora, Emilia flammea, Tripleurospermum maritimum* and *Steirodiscus tagetes.*
Category IV	Crystals are distributed in epicarpic zone of pericarp and testal zone; viz., *Buphthalmum salicifolium.*
Category V	Crystals are distributed in epicarpic and mesocarpic zone of pericarp; viz., *Tanacetum macrophyllum.*

Among the five categories, most prevalent is first category and least common are forth and fifth categories, whereas second and third categories are intermediate in frequency.

In first category, crystals are distributed either in one row (*Anthemis, Arctium, Bothriocline, Gaillardia* and *Vernonanthura*) or in many rows in other taxa. Sometimes crystals are restricted to a specific region of mesocarpic zone. For example, in *Brachycome campylocarpa,* crystals occur in anterio-posterior lobes region, in other taxa in furrow region (*Catananche, Elephantopus, Vernonia cistifolia*) or rib region (*Linzia* and *Vernonia petersii*). In *Baccharoides calvoana* ssp. *meridionalis*, the crystals are found only on the basal arm of inverted 'T'-shaped sclerenchyma bundle of mesocarp. Regarding distribution of crystals, one diacritical thing is the position or location of crystals. In *Anthemis,* these are always found towards inner wall of cells, whereas in *Bothriocline* and *Gaillardia,* they are towards outer wall of cells.

In the second category, crystals are arranged in one row of cells. Secondary sculpture is formed by calcium oxalate crystals, which exist inside the cell, and are visible in SEM pictures as well as cross sections of mature cypselas.

In the third category, crystals are found in definitely organized testal cells as in *Cirsium, Emilia* and *Tripleurospermum*, or in crusted disorganized zone (*Craspedia, Steirodiscus*).

According to Dormer (1961), calcium oxalate crystals occur in the inner part of ovary wall. Our study indicates that this statement is true for the tribe Cardueae, but is not valid for all other taxa of Compositae.

The observations by Källersjö (1985, 1990) on presence or absence of druses in the pericarp in some members of Compositae has a significant value for the tribe Anthemideae. PRYCHID & RUDALL (1999) mentioned that druses are frequently occurring in dicotyledons, whereas raphides are commonly distributed in monocotyledons. The present study does not fit well with this statement, since the majority of the studied species have prismatic crystals and in the Compositae druses or raphides are less common, (reported e.g. from the *Gynura-Solanecio* clade, JEFFREY 1986).

Other than ovary wall, crystals have been noted from floral parts of two species of *Helianthus* (*H. annuus, H. tuberosus)* by MERIC & DANE (2004). We have noted the absence of calcium oxalate crystals in the mature cypselar wall in *Helianthus annuus* (recorded as a taxon without CaOx crystals; Table 2).

Observation on calcium oxalate crystals in various tissues of cypselas in the tribe Cardueae (ZAREMBO & BOYKO 2008) shows that inner region of mesocarp, adjacent to testa in *Serratula*, possesses crystals. Similarly, the border between pericarp and testa in *Synurus* contains crystals. Identical type of distribution of oxalate crystals is seen here in *Anthemis, Arctium* and *Baccharoides.*

Presence of calcium oxalate crystals in the vegetative parts of *Aster squamatus* was recorded by MERIC (2009).

Although morphology and distribution of calcium oxalate crystals are now somewhat known, their functional aspects are not clearly known or understood. There are various opinions regarding the possible functions of oxalate crystals as follows:

i) to regulate the calcium levels in plant cells and tissues (FRANCESCHI & NAKATA 2005).

ii) to enhance the strength of the plant tissue or tissues (FRANCESCHI & HORNER 1980).

iii) to protect against animals especially herbivores (MOLANO-FLORES 2001).

iv) to act as storage tissue of calcium and oxalic acid (FRANCESCHI & HORNER 1980, PRYCHID & RUDALL 1999).

v) to detoxify heavy metals (NAKATA 2003).

vi) to precipitate calcium salt in same specific environmental condition (WHITE & BROADLEY 2003).

vii) to remove toxic substances (BORCHERT 1984).

viii) to overcome salt stress and homeostasis (HURKMAN & TANAKA 1996).

ix) to regulate light intensity in plant tissues (FRANCESCHI & HORNER 1980).

x) to provide mechanical support to the plant tissues (Nakata 2003).

xi) to participate in transformation of light energy to the chloroplast of the parenchyma cells of leaves during photosynthetic process (Kuo-Huang et al. 2007) and

xii) to facilitate pollination by providing a visual signal or a scent interesting to insect (Chase & Peacor 1987, D'Arcy et al. 1996).

Some of these proposed functions are closely related or near synonymous, and some may seem more plausible than others. One recent study (Jáuregui-Zúñiga et al. 2005) suggests that CaOx crystals are important in bulk calcium regulation but not in metal detoxification. Whatever their functions may be, the distribution, shape and structure of calcium oxalate crystals are very specific and obviously taxonomically useful at least in certain plant families (Molano-Flores 2001). From the taxonomic viewpoint, it is worth noting that they are not universally present in all plant organs, but instead they are confined to specific crystalliferous tissue only. Their morphology and distribution have been indicated as genetically controlled by the cell (Ilarslan et al. 2001, Nakata & McConn 2000). Usually a specific taxon possesses a specific type of crystals (Prychid & Rudall 1999). The present study shows similar results. Although crystal formation is gene controlled, various external factors such as temperature, air pressure, light intensity, pH of soil and other environmental factors may affect the formation of calcium oxalate crystals (Franceschi & Horner 1980, Garty et al. 2002, Kuo-Huang et al. 2007, Meric 2008, 2009). Ilarslan et al. (1997, 2001) have also suggested that deposition of calcium oxalate crystals serves as a storage source for calcium and it is assumed that both the mitosis and cytokinesis are regulated by calcium ions (Hepler & Wayne 1985). The climatic condition of an area associated with phenology controls the formation of calcium oxalate crystals in the cambial zone of *Citharexylum* (Verbenaceae) as has been observed by Marcati & Veronica (2005). They have indicated that an abundance of crystals was observed during water deficit condition, whereas during flowering time associated with rainy season, crystals were rarely observed.

Absence of calcium oxalate crystals in the mature cypsela of non-phytomelanin containing taxa is probably the primitive condition. Presence of calcium oxalate crystals without presence of phytomelanin may be one step evolved condition, and absence of calcium oxalate crystals in phytomelanin bearing taxa may be highest evolved state, since these taxa have been considered as belonging to the most advanced lines in the family (Bremer 1994, 1996, Kadereit & Jeffrey 2007). Evolutionary relationships of crystals within the cypselar wall are diagrammatically shown in Table 3. From this observation it is obvious that formation and distribution of calcium oxalate crystals have an evolutionary role.

Therefore, further detailed critical studies regarding the distribution of calcium oxalate crystals in mature cypselar walls in other taxa of Compositae will help developing an improved classification of the Compositae.

Table 3. Probable evolution of cypselar structure of Compositae in relation to calcium oxalate crystals.

Presence of phytomelanin in pericarp: Absence of CaOx crystals in cypselar wall

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Absence of phytomelanin in pericarp: Presence of CaOx crystals in cypselar wall

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Absence of phytomelanin in pericarp: Absence of CaOx crystals in cypselar wall

Acknowledgements

The authors are highly grateful to the Directors and Curators of the following herbaria: AD (Adelaide, South Australia); BRI (Queensland, Australia); LISC (Lisboa, Portugal); NSW (Sydney, Australia); RB (Rio de Janeiro, Brazil); SRGH (Harare, Zimbabwe); and Z (Zurich, Switzerland) who kindly have provided mature cypselas for this study.

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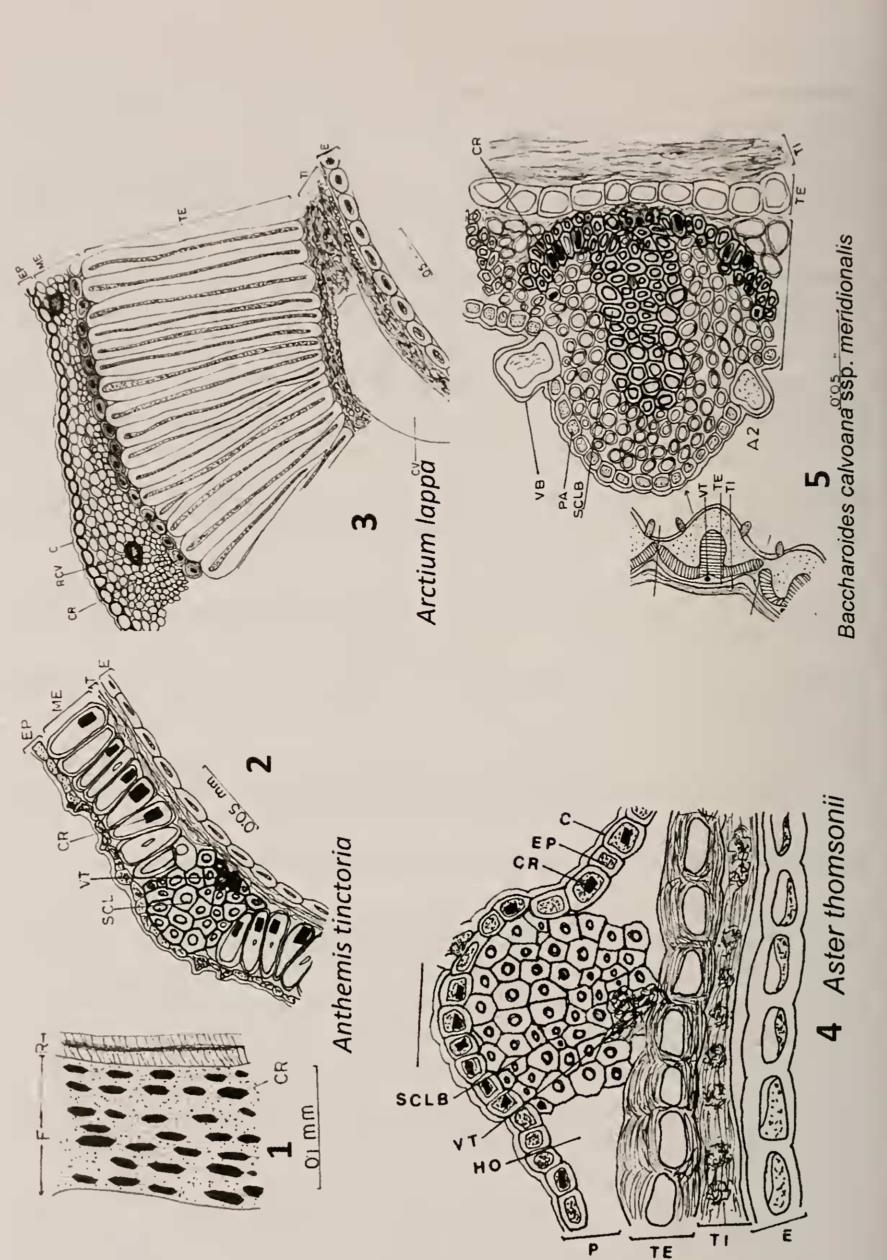
Figs. 1–27. Distribution of calcium oxalate crystals in different taxa.

1,2: *Anthemis tinctoria*. 3: *Arctium lappa*. 4: *Aster thomsonii*. 5: *Bothriocline laxa*. 6: *Brachycome campylocarpa*. 7: *Buphthalmum salicifolium*. 8: *Carpesium cernuum*. 9: *C. nepalense*. 10: *Catananche caerulea*. 11: *Cirsium vulgare*. 12: *Craspedia uniflora*. 13: *Elephantopus scaber*. 14: *Emilia flammea*. 15: *Gaillardia aristata*. 16: *Inula ensifolia*. 17: *Linzia melleri*. 18: *Tripleurospermum maritimum*. 19: *Polydora poskeana*. 20: *Steirodiscus tagetes*. 21: *Tanacetum macrophyllum*. 22: *Vernonanthura condensata*. 23: *Vernonia cistifolia*. 24: *Baccharoides calvoana* ssp. *meridionalis*. 25: *Vernonia petersii*. 26: *V. senegalensis*. 27: *Buphthalmum speciosissimum*.

(C - Cuticle. CR - Crystal. D - Druse. E - Endosperm. EP – Epicarp. HO- Hollow. ME - Mesocarp. NCP - Non Cellular Pellicle. P - Pericarp. PA - Parenchyma. PH – Papillate Hair. RCV – Resin cavity or Resin duct. SC – Sclerosed cells. SCC – Secretory cells. SCL - Sclerenchyma. SCLB – Sclerenchyma brace or bundle. SCV – Secretory cavity. T - Testa. TE – Testa epidermis. TI – Testal inner zone. TM – Testal middle zone. TPA - Thin-walled parenchyma. TWPA- Thick-walled parenchyma. VB – Vesicular body. VT - Vascular trace or vascular tissue).


1
0.1 mm
F
R
CR
Anthemis tinctoria
2
0.05 mm
EP
ME
T
E
CR
VT
SCL
3
Arctium lappa
EP
ME
TE
TI
E
C
RCV
CR
CV
0.5
4
Aster thomsonii
C
EP
CR
SCLB
VT
HO
P
TE
TI
E
5
Baccharoides calvoana ssp. meridionalis
CR
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VB
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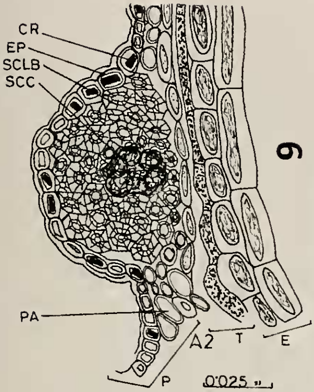
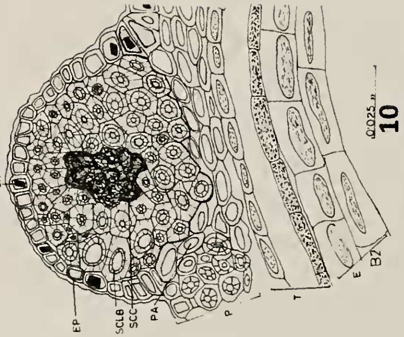
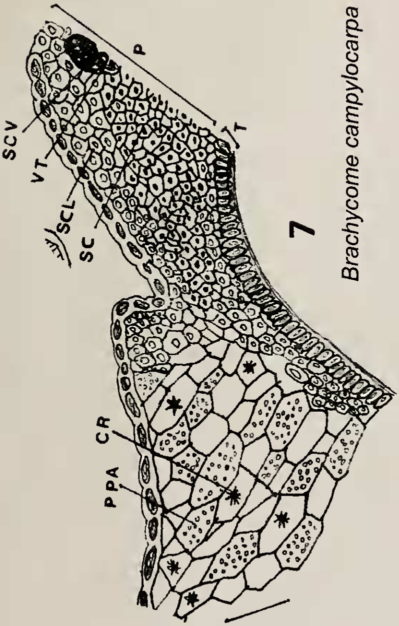
TE
TI
A2
0.05



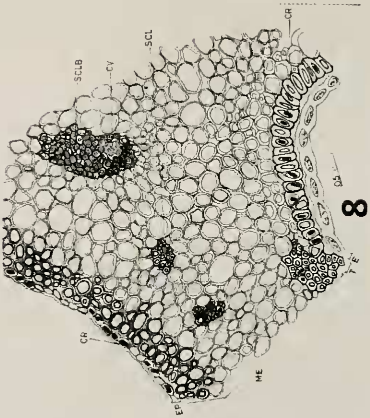
Carpesium cernuum



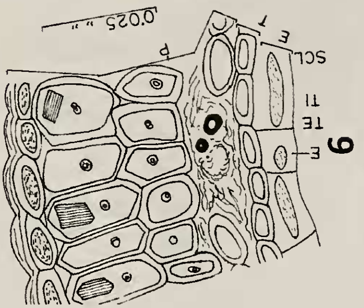
Carpesium nepalense



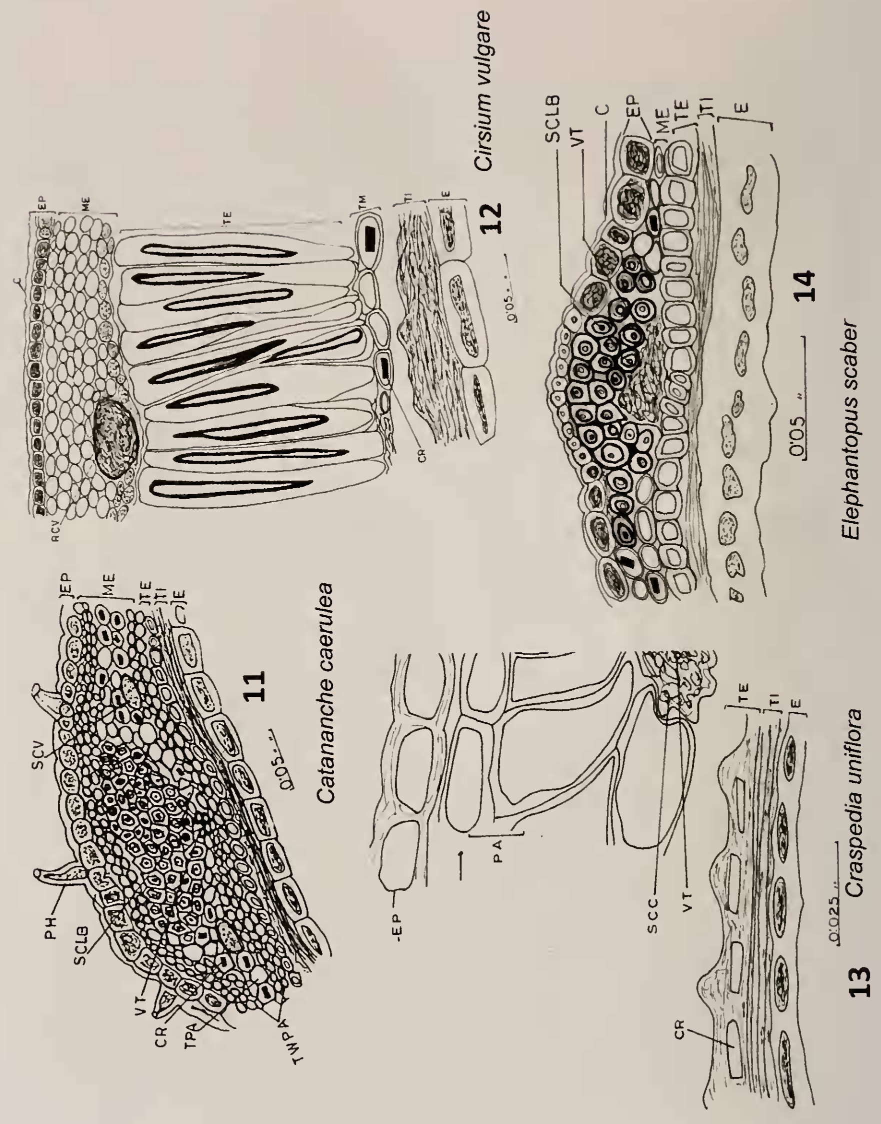
Brachycome campylocarpa



Buphthalmum salicifolium



Bothriocline laxa ssp. *laxa*


11
Catananche caerulea
SCV
PH
SCLB
VT
CR
TPA
TWPA
EP
ME
TE
TI
E
12
Cirsium vulgare
RCV
TM
CR
13
Craspedia uniflora
EP
PA
SCC
VT
CR
TE
TI
E
14
Elephantopus scaber
SCLB
VT
C
EP
ME
TE
TI
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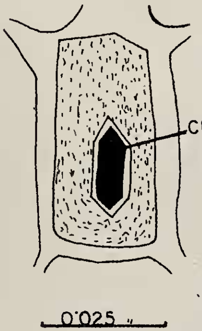
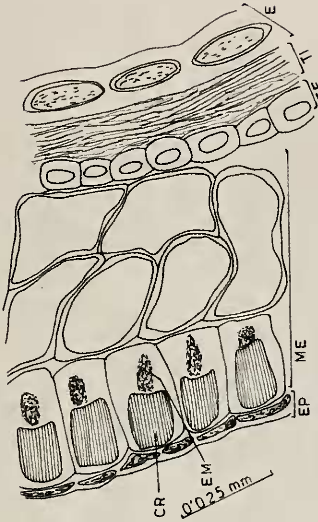
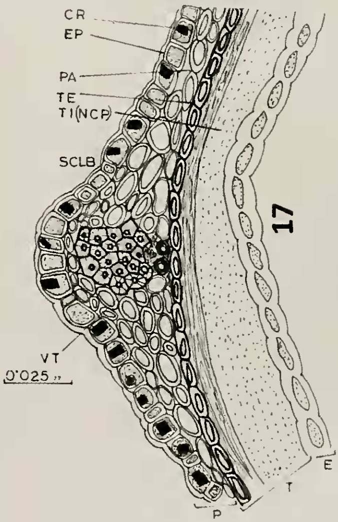


15 *Emilia flammea*

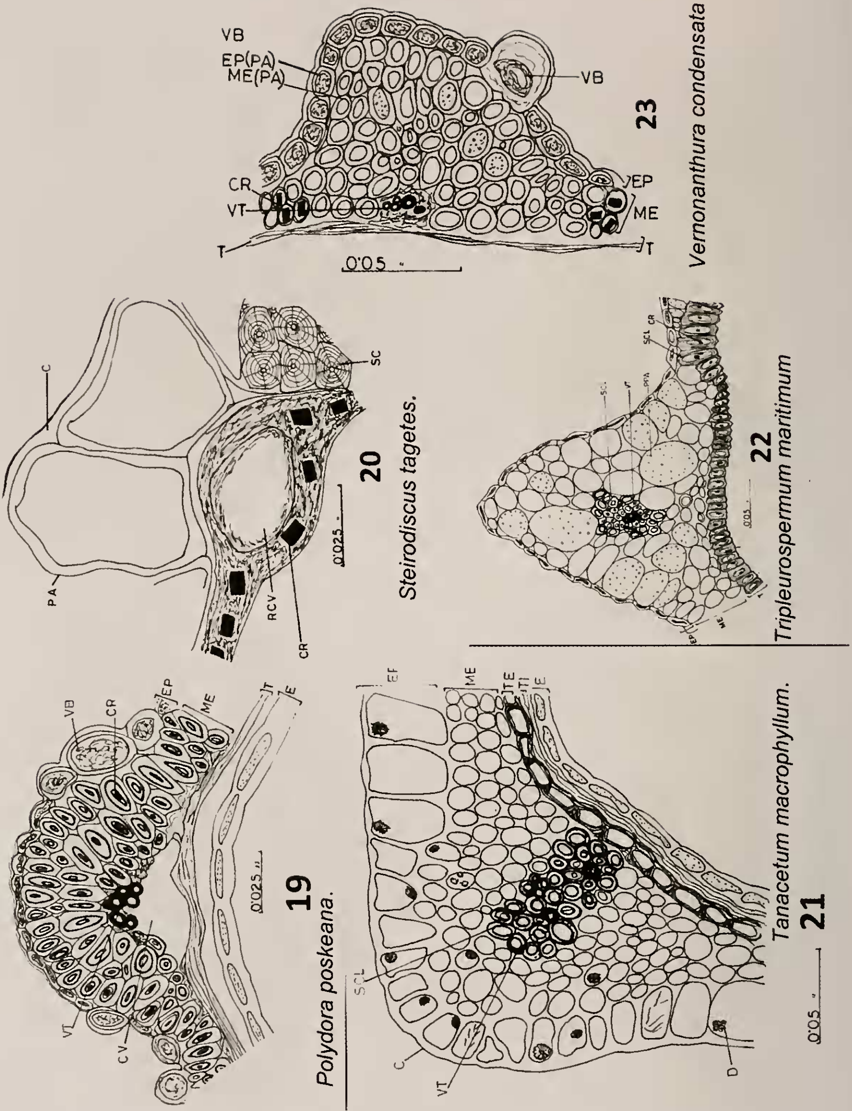


16 *Gaillardia aristata*



17 *Inula ensifolia*

18 *Linzia melleri*



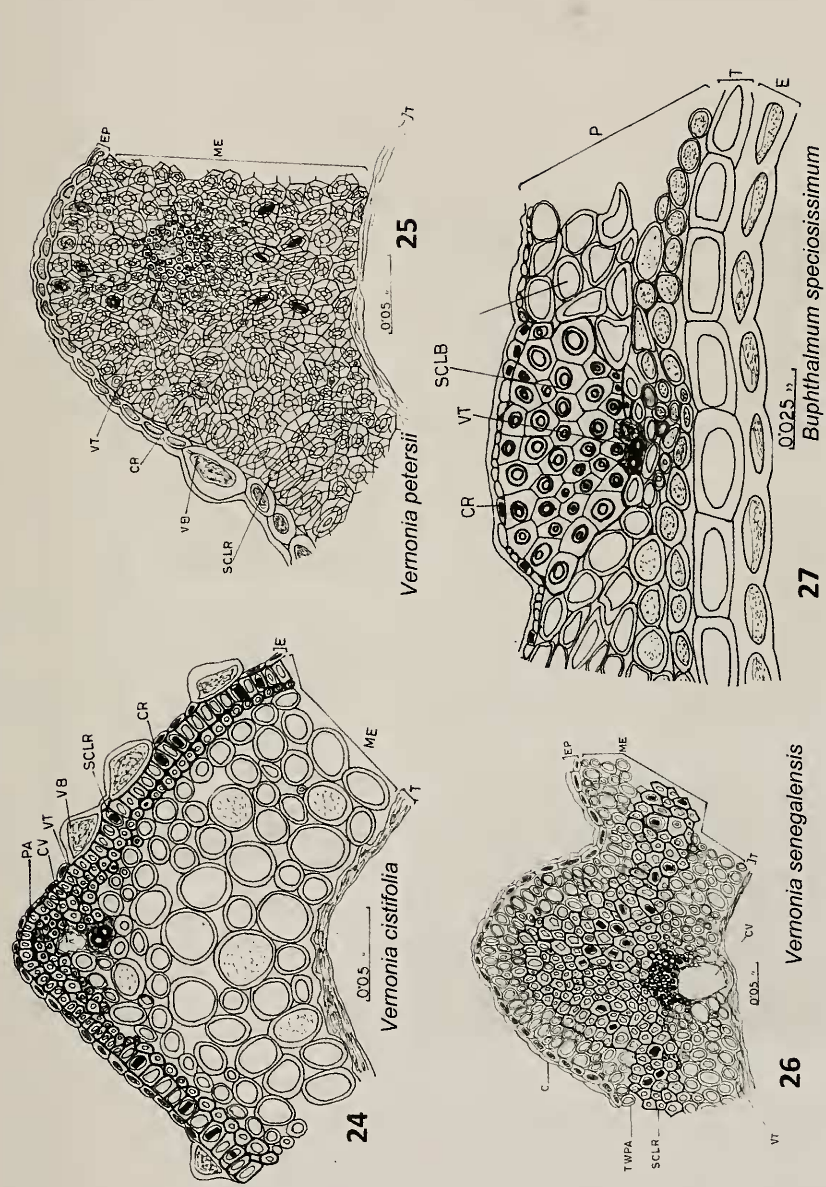
19 *Polydora poskeana.*

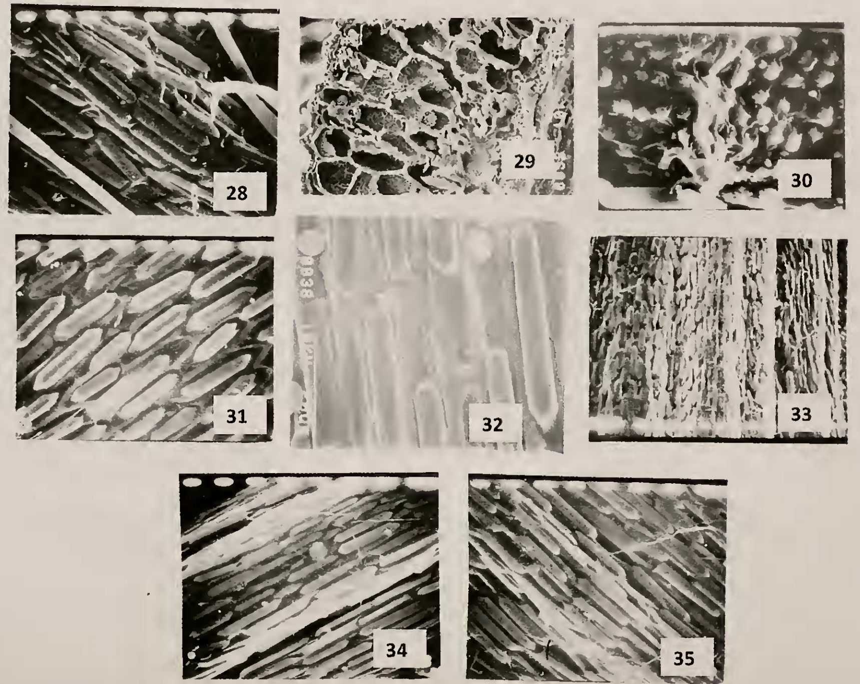
20 *Steirodiscus tagetes.*

21 *Tanacetum macrophyllum.*

22 *Tripleurospermum maritimum*

23 *Vernonanthura condensata*


24
Vernonia cistifolia
25
Vernonia petersii
26
Vernonia senegalensis
27
Buphthalmum speciosissimum



Figs. 28–35. SEM photographs showing the distribution of calcium oxalate crystals.

28: *Aster thomsonii*, × 800. 29: *Brachycome campylocarpa*. 30: *Brachycome heterodonta* var. *heterodonta*, × 1600. 31: *Buphthalmum salicifolium*, × 400. 32: *Buphthalmum speciosissimum*. 33: *Carpesium cernuum*, × 800. 34: *Carpesium nepalense*, × 800. 35: *Inula ensifolia*, × 800.